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Radiation-induced damage in the central nervous system: An interpretation of target cell responses

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Effects of irradiation on the central nervous system (CNS) have received much interest since the early 1930s, when experimental studies on monkey and dog brain were started (see later). One of the features which prompted so much interest was the observation of a long delay time before any clinical signs of damage developed. During the early 1960s, at least three large conferences were devoted to the subject of effects of ionizing radiation on the nervous system (e.g., IAEA, Vienna, 1961). In the light of the present conference, it is perhaps of interest to quote from the work of Zeman *et al.* (1964), who were among the first to perform detailed studies on the pathogenesis of late radiation damage in the rat and monkey spinal cord. These authors stated, after a remark on the absence of radiobiologists in the field of radiation pathology: 'Perhaps it is wise to take this hint of the scientifically well-equipped radiobiologist and to stay clear from an area in which an incalculable array of variables makes clear-cut experimentation a hopeless venture'.

In the present report, 20 years and many experiments later, the contribution of radiobiological studies to the understanding of late radiation-induced damage in the CNS is reviewed, and an attempt is made to combine some of the results obtained by Zeman and co-workers (1964) with more recent data into a model of target cell responses in the CNS. However, it still seems too early for any radiobiologist to claim the award offered by O.T. Bailey (1962) for an explanation of the processes taking place during the latent period before delayed radiation necrosis of the CNS develops.

Tissue architecture and cellular kinetics

The nervous system is anatomically divided into a central part (CNS), consisting of brain and spinal cord, and a peripheral part (PNS), which is the collection of all cranial and peripheral nerves outside the protective environment of the skull and the vertebral bones. Internally, the CNS is further protected by the blood-brain barrier, which very effectively limits the permeation of predominantly large hydrophilic molecules from the bloodstream into the parenchyma. This characteristic of the CNS is employed, e.g. in cancer chemotherapy, where several effective drugs such as cis-platin, vincristin and adriamycin are extremely neurotoxic if directly administered into the CNS. Radiation doses in the therapeutic range have been shown experimentally to change the permeability of the barrier to drugs such as methotrexate (Griffin *et al.*, 1977; Storm *et al.*, 1985) at least temporarily, but no general evidence exists on enhanced neurotoxicity of drugs after radiation treatment of the CNS.

The number of parenchymal cell types in the nervous system is rather limited, and consists of 2 major classes, both of neuroectodermal origin, the neurons and the supportive glial cells. Many types and classes of neurons exist, but they all share one important radiobiological characteristic in that they stop proliferation before or shortly after birth. In rats, the longest post-natal period during which some neuronal cell types continue to proliferate is 3 weeks, but the majority ceases proliferation before birth (see review by Schultze & Korr, 1981).

The other group of cells of neuroectodermal origin, the glial cells, can be divided into two major types, astrocytes and oligodendrocytes. Even in the mature stage of development, these cells retain their ability to revert to a mitotic state, albeit the turnover in the undisturbed tissue is extremely slow. *Astrocytes* are thought to provide a 'protective shielding' of neurons, and are the primary cell type involved in tissue repair and scar formation. Fibroblasts are absent in the CNS, so that the typical late radiation fibrosis, as seen in most other tissues, does not develop. *Oligodendrocytes* are the glial cells involved in the formation and maintenance of the myelin sheath, the characteristic membranous structure that is responsible for the efficient high-speed nerve-pulse propagation in higher organisms. The integrity of the myelin sheath depends on the presence of oligodendrocytes, and loss of these cells leads to so-called segmental demyelination, leaving a bare, unprotected axon. As long as the axon is intact,

remyelination may occur by proliferation of glial cells and the formation of characteristically thin, short segments. Also, Schwann cells may invade from the adjacent nerve roots and participate in remyelination, as has been described after local irradiation of the neonatal spinal cord (Gilmore, 1971; Blakemore & Patterson, 1975).

Schwann cells have a different embryonal origin from glial cells, and are the primary cell type in the peripheral nervous system, involved in myelination and regeneration of spinal nerve roots and peripheral nerves. Each Schwann cell is part of only one myelin segment, in contrast to oligodendrocytes which through cytoplasmic processes are connected to as many as 50 myelin segments (Peters & Vaughn, 1970). Thus, per individual cell, the loss of oligodendrocytes may cause a more extensive demyelination as compared to Schwann cells.

A cell type that is usually classified as a glial cell is the microglia. Since its discovery by the famous Spanish school of neuropathologists in the early 1920s (del Rio-Hortega), its origin and function has been much disputed. Even today the origin of the microglial cell is not known, but it is thought to act primarily as a macrophage in minor trauma of the CNS. Microglia has also been implicated as a stem cell, or glioblast, for the major two glial cell types.

The cellular kinetics of glial cells have been extensively studied, and recently reviewed by Korr, Schultze and co-workers (1975, 1981). From their own results, and an extensive review of the literature (Schultze & Korr, 1981) these authors concluded that glial and endothelial cells continue to proliferate during adult life. The cell cycle time of proliferating cells is $\sim$20 h, and the growth fraction declines from $\sim$10% in the young, 2-week-old rat to 0.4% in the brain of the adult mouse. Approximately half of the newly produced cells leave the growth fraction, but a small fraction of non-proliferating cells re-enters the growth fraction (Korr *et al.*, 1983). This shows that differentiated glial cells retain the capability to revert to the mitotic state, and an increased proportion may do so when cells are lost after a cytotoxic insult.

The proliferation of glial cells *in situ* has been reported to be similar in different regions of the brain, and is most likely independent of the proliferative activity in the subependymal plate (Korr *et al.*, 1983). The subependymal plate is a vestige from the embryonal development of the brain, which remains mitotically active throughout adult life. This structure is the only part of the CNS in which acute cellular responses to ionizing radiation can be quantitated (e.g. Hubbard & Hopewell, 1980), but the relationship between these acute changes and the various types of late damage in the brain is not well established. The cellular kinetics of the subependymal plate, and the effects of irradiation, have been reviewed recently (van der Kogel, 1983).

The most detailed studies of glial and endothelial cell kinetics in the CNS have been carried out in mouse and rat brain. For the spinal cord, no direct measurements of cell cycle parameters have been reported, but labelling indices in the cord were not different from those in the brain (Hubbard & Hopewell, 1979; Hornsey *et al.*, 1981). In view of the similarity in tissue architecture between cord and brain, and the absence of a subependymal layer in the cord, it seems reasonable to assume similar mechanisms of cell renewal in cord and brain.

Functional and histological assays of damage

Radiation-induced damage in the central nervous system is characterized by the long duration of latency intervals, before major functional impairment is observed. One of the primary types of damage, described in many different species including man, has been called delayed radionecrosis since the early experimental studies in dogs (Scholtz, 1934), monkeys (Davidoff *et al.*, 1938) and rabbits (Russell *et al.*, 1949). This type of damage occurs both in the brain and in the spinal cord after a latency interval of 4–6 months, and has a predilection for white matter. Ever since the first reports on this syndrome, opinions about its pathogenesis have been divided between a primary glial and a primary vascular origin. The occurrence of various syndromes of radiation damage in the CNS has been the subject of various reviews (Hopewell, 1979; van der Kogel, 1983), and are briefly summarized in the present paper as far as pertinent to the discussion of target cell responses.

For the various radiation-induced lesions in the CNS, a close similarity is observed between brain and spinal cord. However, because of its anatomical structure, lesions in the spinal cord are more directly correlated with the development of various degrees of neurological impairment. This correlation is less apparent in the brain, where lesions in several areas are not accompanied by signs of neurological damage, or are indirectly expressed by weight loss or sudden death. Therefore, the occurrence of various types of damage will be mainly described for the spinal cord.

Perhaps the only type of damage which is not seen in the spinal cord, is the acute effect of radiation on the cells of the subependymal plate. This is the only region in the CNS in which an immediate cellular response can be measured after irradiation, and for which cell survival curves could be obtained (e.g. Hubbard & Hopewell, 1980). It has been hypothesized that a correlation exists between these acute cellular changes in a glial stem

cell population, and the delayed white matter necrosis (Hopewell, 1979).

The earliest sign of damage which occurs more diffusely throughout the white matter, is the occurrence of nodal widening and segmental demyelination, as early as two weeks after doses of 5–60 Gy (Mastaglia *et al.*, 1976). Segmental demyelination is a direct expression of glial cell damage, and shows a dose-dependent progression. After about 2 months, remyelination was observed to start. In rodents this type of damage is not accompanied by functional impairment, but in human patients this diffuse demyelination is reflected by the occurrence of Lhermitte's sign of electrical paraesthesias after cord irradiation, and a somnolence syndrome after brain irradiation.

After this 'latent period' of about 4–6 months, distinct areas of necrosis develop in the white matter, which in the spinal cord is reflected by a usually rapidly progressive paresis or paralysis. The rapid progression from focal demyelination into areas devoid of glial nuclei, followed by tissue necrosis, has led many authors to the conclusion of a primary glial origin (reviewed by Hopewell, 1979; van der Kogel, 1983). Other authors concluded that the large areas of necrosis cannot be purely the result of loss of oligodendrocytes, but that vascular damage must play an important role (Blakemore & Palmer, 1982). A complicating factor which confuses the issue of pathogenesis, is the dose-dependency of different lesions. The threshold dose for white matter necrosis in most species is in the range of 20–25 Gy (single dose). With increasing dose, the latent period generally decreases, and vascular damage (mostly haemorrhages) becomes more pronounced (Knowles, 1983; van der Kogel, 1979). This suggests a precipitous role of damage of the vascular system in the expression of predominantly glial cell damage (see Figure 1).

A different type of vascular damage, not associated with white matter necrosis, is observed after very long time intervals in the rat cervical spinal cord, up to the end of the normal life-span of about two years. The lesions observed vary considerably, from telangiectasia and petechial haemorrhage in neurologically normal rats, to extensive haemorrhagic infarcts in acutely paralyzed rats. The use of this endpoint in radiobiological studies is based on a histological scoring system (van der Kogel, 1979). Similar lesions have been observed in the brain after long latent periods (Reinhold & Hopewell, 1980). In the guinea pig lumbar spinal cord, after doses below the threshold for white matter necrosis, no vascular damage was noted, but there was a diffuse vacuolar demyelination.

Finally, a specific type of damage is observed in the lumbosacral region of the rat spinal cord (cauda equina). Even after single doses as high as 40 Gy, no spinal cord necrosis was seen, but damage was

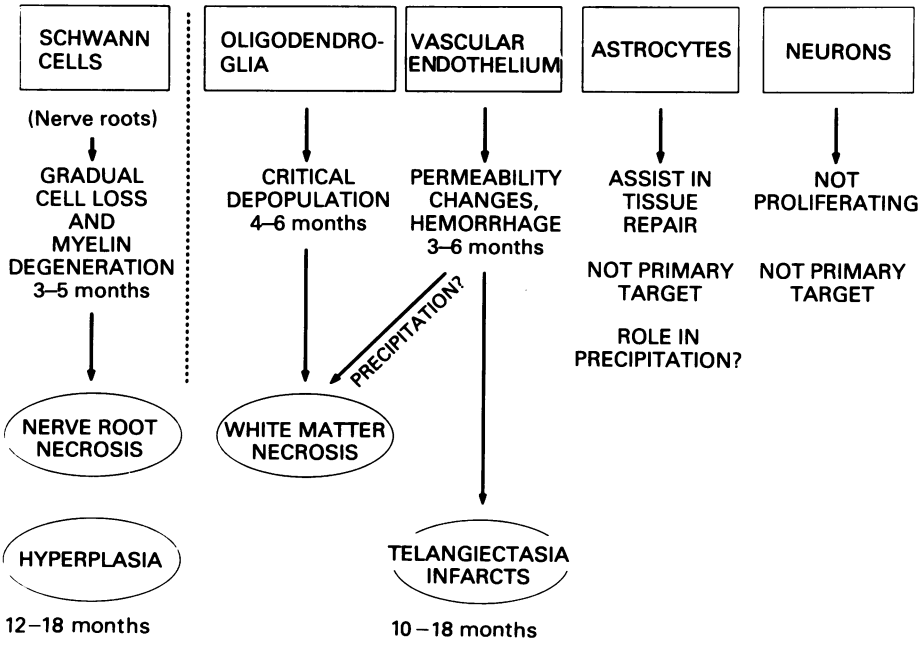

Figure 1 A schematic representation of the major cell types in the CNS, and their assumed participation in the development of different types of radiation-induced lesions. Schwann cells (on the left) are not part of the CNS, but are the primary parenchymal cells in the spinal nerve roots.

restricted to a gradually developing demyelination and necrosis of the nerve roots, which is associated with loss of Schwann cells (Bradley *et al.*, 1977; van der Kogel & Barendsen, 1974). In contrast to the sudden onset of white matter necrosis in the higher regions of the cord, nerve root necrosis progressed more gradually over a period of several months. The process of nerve root degeneration appeared to occur largely independent of dose in the WAG/Rij strain, but a more dose-dependent progression was seen in the BN/Bi strain of rats (van der Kogel, 1979). This observation was reflected in the dose latent-period relationships for hindleg paresis.

Dose latent-period relationships

The length of the so-called latent period and its dependence on dose, has been a subject of considerable interest ever since the first reports on delayed radionecrosis of the brain. Some of the earliest studies on radiation-induced brain damage which showed a reduction in latent period were performed in large animals such as rabbits (Russell *et al.*, 1949), dogs (Scholz, 1934) and rhesus monkeys (Davidoff *et al.*, 1938). Similar observations were made on the spinal cord of large animals, but it was not until the studies by Innes, Carsten and Zeman (1962, 1964) that large scale experimentation with rats and mice on late radiation effects in the CNS was started. As expected, the use of larger numbers of animals from inbred strains gave a better accuracy and reproducibility of results. As mentioned before, effects on the brain were mostly studied in relation to the cellular kinetics of the subependymal plate, although a dose latent-period relationship for rat brain was shown (Hopewell & Wright, 1970). The nonspecific character of signs of functional deficits in the brain of rats or mice (sudden death, weight loss) made it less amenable to analysis in terms of radiation tolerance or dose-latent period relationships. Also, the sensitivity of adjacent structures (oral mucosa, eyes) made it more difficult to irradiate the whole brain including the brain stem of these small animals with a homogeneous dose distribution and keep the animals under observation for the remainder of their lifespan.

The situation is different for the spinal cord, in which radiation induced damage is accompanied by specific neurological signs. Therefore, most experimental data on radiation tolerance and dose/latent-period relationships have been derived for the spinal cord, primarily in rats. In these animals, the decrease in latent period with increasing doses has been well established (Carsten & Zeman, 1966; Hopewell & Wright, 1975), but at the same time has been a matter of controversy. When latent periods were investigated at high doses over a large range (up to 100 Gy in single doses), a linear decrease with increasing dose was observed in mice (Geraci *et al.*, 1974, 1978; Goffinet *et al.*, 1976) and in rats (van der Kogel, 1979). However, when studied over a relatively small dose-range and particularly close to threshold dose levels for permanent damage, dose-latent period relationships showed a more complex pattern (Figure 2). In WAG/Rij rats, doubling the dose from 20 to 40 Gy to the cervical cord reduces the latent period from 170 to 140 days. Although this difference of 30 days is statistically significant, it is much smaller than the variations seen as a function of age or between different strains (see below). For the lumbar region (L2–L4), the dose-latent period relationships show an even more complex pattern, and between 20 and 40 Gy the presence of a minimum latent period was suggested for some strains (White & Hornsey, 1978; van der Kogel & Barendsen, 1974) but not in two other strains (van der Kogel, 1979). However, at higher doses in the lumbar cord the latent period linearly decreases with increasing dose, which is similar in the WAG/Rij and the BN/Bi strain (van der Kogel, 1979).

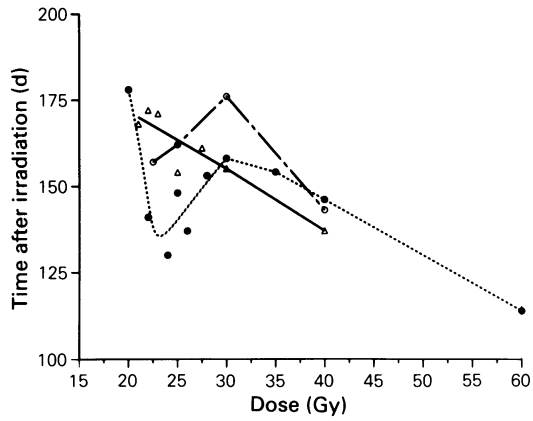

Figure 2 Dose-latent period relationships for different regions of the rat spinal cord. WAG/Rij rats; 300 kV X-rays. (△) C_5-T_2; (○) $T_{12}-L_2$; (●) L_2-L_4.

The presence of these complexities in dose-latent period relationships, especially when studied over a limited dose-range, has led to differences in interpretation and seemingly controversial conclusions (Masuda *et al.*, 1977; Hubbard & Hopewell, 1978; Schultheiss *et al.*, 1984). These observations also imply that one should be extremely cautious in using the latent period as an endpoint in determining dose-modifying factors (such as for hyperbaric oxygen or chemotherapeutic drugs) or in

establishing RBE values for high LET radiation. The latent period is obviously a reflection of the kinetics of functional cell loss. In addition to changes in the rate of cell loss, it is also possible that a decreasing latent period represents a shift in the interaction between several target cell populations. As discussed before, the more conspicuous vascular damage at higher doses in the cervical cord may precipitate the damage in the primary target cell population, the oligodendrocytes (Figure 1). After lower doses, and at much longer times after irradiation (9–18 months), a different type of vascular damage is seen in the absence of the typical myelin-related lesions. The latent period at the ED_{100} level for the early delayed response (white matter necrosis) has been found to be highly reproducible for inbred strains of rats of a particular age and sex. This is in contrast to the late vascular response, which shows a large variability. The strong correlation between dose and latent period for white matter necrosis, and its small variability among rats within one inbred strain, indicates a well programmed chain of events. This aspect will be further discussed in the analysis of long term repair of residual damage.

It is interesting to note, that in different rat strains studied, the latent periods at isoeffect doses range from ~120–200 days (Table I). Apparently, small differences in cell kinetic parameters are magnified by the time a critical surviving cell number is reached and tissue breakdown occurs. Within the same strain (WAG/Rij), a significant reduction in latent period was seen when rats were 5 weeks old at the time of irradiation. At the ED_{100} isoeffective dose, the latent period for adult rats was 170 days, which was reduced to 100 days for 5-week-old rats. A similar observation was made in 30-day-old guinea pigs (Knowles, 1983). At an age of 5 weeks, although myelination is complete, a larger fraction of cells may still be in cycle, with a more rapid turnover of functional cells. As was shown by Korr *et al.* (1983), the growth fraction of glial cells in the brain of 2-week-old rats is 4–12%, in contrast to 0.4% in adult mice (Korr *et al.*, 1975).

The significant reduction in latent period for immature animals is not accompanied by an equally large reduction in isoeffect dose for white matter necrosis. For irradiations with single doses or 10 fractions, the ED_{50} was at most 5–10% lower for young animals, which was not statistically significant (van der Kogel, unpublished results). This suggests that in an immature, 5-week-old rat the number of target cells at risk is not smaller, nor are they more sensitive, but expression of damage is faster when compared to young adult rats. This agrees with the faster turnover rate of glial cells in the immature rats.

Dose-response relationships

For the various endpoints described, but predominantly for the occurrence of white matter necrosis in cervical or thoracic spinal cord, dose-response relationships have been reported. In most studies, the development of paralysis within one year after irradiation correlated well with the histological lesion of white matter necrosis. Because of the quantal aspect of the endpoints used, dose-response curves were usually obtained by probit analysis, from which effective doses can be derived at different levels of response (e.g. ED_{50} or ED_{100}). It is important to realize that these values do not reflect different levels of injury, but the probability of inducing a specific lesion (white matter necrosis)

Table I Latent periods for different regions of the rat spinal cord at ED_{100} dose levels

Region of cord	*Radiation*	*Strain (sex)*	[a]*ED_{100} (Gy)*	*Latent period (days)*	*Reference*
C2–T1	18 MeV X	WAG/Rij(M)	24	166±8	[b]
C5–T2	300 kV X	WAG/Rij(M)	21	170±10	van der Kogel (1979)
C5–T2	300 kV X	BN/Bi(M)	24	198±17	van der Kogel (1979)
C2–T2	300 kV X	F344(F)	18	120±5	[b]
T9–T11	Co-γ	SD(F)	38(2fr)	185±30	Masuda *et al.* (1977)
T12–L1	Helium-ions	CD-1(M)	~25	160±23	Leith *et al.* (1975)
T12–L2	300 kV X	WAG/Rij(M)	22.5	157±28	van der Kogel (1979
L2–L4	300 kV X	WAG/Rij(M)	20	178±37	van der Kogel (1979)
L2–L4	300 kV X	BN/Bi(M)	24	200±39	van der Kogel (1979)
L3–L5	250 kV X	CFHB(M)	25	150±20	White & Hornsey (1978)

[a]ED_{100}: Derived from dose-response curves or represents the lowest dose at which 100% of the animals are paralyzed.
[b]Unpublished results.

at different doses. The dose-response curves are usually very steep, and typically rise from 0–100% incidence of paralysis with a 5–10% increase in dose. Generally, ED_{50} values are used when comparing isoeffective doses for different types of treatments. During the last decade, extensive information has been obtained on different variables which influence the radiation response. This includes: effects of high-LET radiation (Leith *et al.*, 1982; White & Hornsey, 1980; van der Kogel, 1985), effects of multi-fractionation (Hornsey & White, 1980; Ang *et al.*, 1983, 1985; van der Kogel & Sissingh, 1983), kinetics of repair of sublethal damage (Ang *et al.*, 1984), and long term repair of residual damage (Hornsey *et al.*, 1982; van der Kogel *et al.*, 1982).

The fractionation studies have shown a large capacity of the spinal cord to repair sublethal injury, resulting in a steep increase in isoeffective doses when the fraction size was reduced. Soon after the linear-quadratic (LQ) model of cell survival was applied to normal tissue responses (Douglas & Fowler, 1976), it became widely accepted as a method to express repair characteristics of the supposed target cells. This has been the subject of a large number of publications, and was also discussed extensively during the 11th L.H. Gray Conference (Br. J. Cancer, Suppl. VI, 1984). For the present discussion, it suffices to state that α/β values derived for different regions in the spinal cord, range from $\sim$1.5–2.5 Gy for the cervical cord (white matter necrosis), and 3–5 Gy for the lumbar cord (nerve root necrosis) (Leith *et al.*, 1981; van der Kogel, 1985). For the late vascular damage in the cervical cord, an α/β ratio of 2.8 Gy was derived (van der Kogel, 1979). These differences in α/β ratios for different effects in the spinal cord, support the observation of the involvement of different target cells.

Based on the concept of target-cell survival in normal tissue responses, and assuming equal effectiveness at equal fraction sizes, isoeffect survival curves can be derived from multifraction experiments. In Figure 3, isoeffect survival curves are presented for the cervical cord in two rat strains (WAG/Rij and F344), and two types of low-LET radiation. Although the accuracy of the LQ model to predict tissue responses at the high and low end of the survival curves is still questionable (Ang *et al.*, 1985), it has the advantage of simplicity and a sound radiobiological basis.

A useful application of the LQ model is the possibility to calculate the effectiveness of part of a treatment, e.g. half of a split-dose course or an individual dose fraction. This concept has been formalized for tissue responses by Barendsen (1982). A basic idea of this concept is the

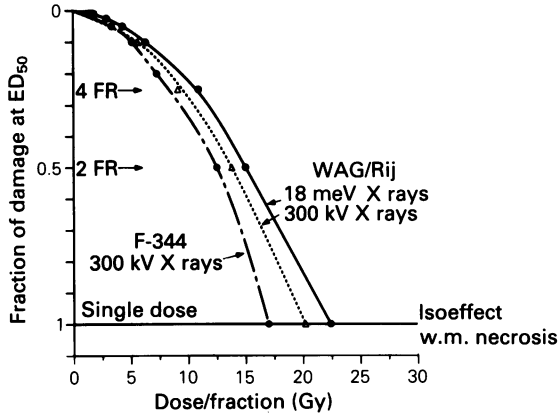

Figure 3 Isoeffect survival curves for the induction of white matter necrosis after irradiation of the cervical spinal cord of two rat strains (WAG/Rij and F344). For construction of the curves, an equal effectiveness per dose fraction is assumed.

comparison of different fractionation schedules by calculation of an extrapolated tolerance (or total) dose (ETD). When the tolerance dose (D_n) is known for a certain fraction size (d_n), as well as the α/β ratio for a particular response (e.g. 2 Gy for white matter necrosis), the mathematical formalism is (Barendsen, 1982):

$$\text{ETD} = D_n\{1 + d_n/(\alpha/\beta)\} \quad (1)$$

As can be seen from formula (1), ETD approaches D_n, when d_n approaches 0. In other words, ETD is the tolerance dose at infinitely small fraction sizes. Once the ETD is known, tolerance doses for other fraction sizes can be calculated. For normal tissue responses in experimental animals, D_n usually represents the ED_{50} dose.

Long term repair of residual injury

The ETD concept has been used in the present paper to calculate the effectiveness of different doses per fraction in experiments on long term recovery of residual injury in the spinal cord. Long term recovery in the spinal cord has been reported for the lumbar cord (White & Hornsey, 1980; Hornsey *et al.*, 1982; van der Kogel, 1979) and the cervical cord (White & Hornsey, 1980; van der Kogel, 1980; van der Kogel *et al.*, 1982). All of these experiments were performed as split-dose, 2 fraction irradiations with various time intervals. The results were analyzed by expressing the ED_{50} obtained for various intervals, relative to the ED_{50}

for a one-day interval. However, since with increasing ED_{50} the size of each dose fraction increases as well, this analysis does not take into account the increased effectiveness of each dose fraction. These data have been reanalyzed, and the effectiveness of each half of a split-course treatment was calculated as the percentage of the corresponding ETD. These percentages of the ETD were summated, and the dose-equivalence of long term recovery was expressed as the percent increase in ETD. The results for the cervical cord are presented in Figure 4. The lower part of Figure 4 shows the percent increase of the ETD for the total treatment. After a time-lag of ~60 days, the ETD is observed to increase, until it reaches a maximum of 135%, compared to an ETD for similar fraction sizes and an interval of one day. In the upper part of Figure 4, the latent periods (at ED_{100} dose levels) calculated from the time of the first dose, show a pattern which parallels the increase in isoeffective doses, but the occurrence of a plateau is not observed. The relationship between latent period (L) and time interval (T) between two fractions can be expressed as:

$$L_{2fr/T} = 170 \qquad (\text{for } T < 60d) \qquad (2)$$

$$L_{2fr/T} = 170 + (T - 60) \qquad (\text{for } T > 60d) \qquad (3)$$

Also in Figure 4, preliminary results are presented from multifraction experiments on long term repair of residual injury. In these experiments the dose per fraction was kept constant at 1/10 of the ED_{50} for a 10 fraction treatment. The initial treatment consisted of 5 (50% ED_{50}), 7 (70% ED_{50}), or 9 (90% ED_{50}) fractions. Because of the constant dose per fraction, the effectiveness of each fraction was known to be 10% of the corresponding ED_{50}. After intervals of 105 and 210 days, a variable number of dose fractions of the same size as used for the initial treatment, was given as a test dose. The total ED_{50} for both treatments was expressed as a percent increase of the ETD (or $ED_{50/10fr}$). The tentative results indicate, that the amount of long term repair depends on the size of the first treatment. Also, the latent period at the ED_{100} dose level shows a similar dependence on the size of the first dose. The relevance of these results for the interpretation of cellular mechanisms of damage is discussed later.

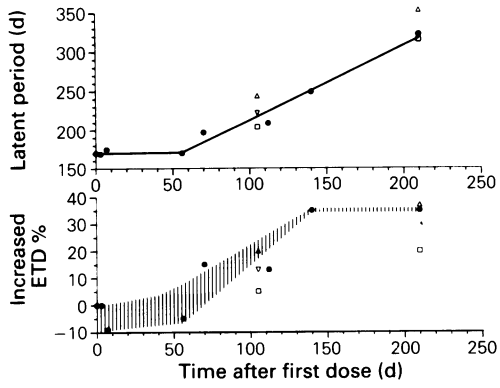

Figure 4 Dose-latent period relationships (upper curve) and the increase of the total isoeffect dose as a percent increase of the ETD (lower curve), as a function of time after the first dose of a split-dose treatment (●). The open symbols represent a multifraction, split-dose treatment with a fixed fraction size, based on the ED_{50} for a 10 fraction irradiation. The number of fractions of the first treatment was 5(△); 7(▽); or 9(□); followed by a variable number of fractions in the second treatment as a test dose.

Target cell kinetics and the radiation response

As was discussed before, the most likely organization of glial and endothelial cell proliferation in the CNS (outside the subependymal plate in the brain) is the existence of a small growth fraction, with a permanent exchange between the growth fraction and the large pool of non-proliferating functional cells. Once in cycle, progression occurs at a rate comparable to cycling cells in other tissues (T_c less than 24 h). One of the first extensive cell kinetic studies on the effects of X-rays in the rat spinal cord was reported by Zeman *et al.* (1964). After a single dose of 29–35 Gy on the high thoracic cord (T2–T4), these investigators observed no significant changes in the glial/endothelial cell population during the first 4–5 months post-irradiation (p.i.). At 5–6 months p.i., a sudden increase of cells by about 50–60% occurred shortly before the development of white matter necrosis. The labelling index (LI) at that time increased from the very low control values of ~0.2% to ~10%. Shortly after this drastic increase, labelled cells disappeared within a few days after they had been labelled. Zeman *et al.* (1964) interpreted these observations as being in contrast to the general opinion that 'irradiated cells usually die when they attempt cell division'. In view of the present author, these very thorough studies performed more than 20 years ago by Zeman and co-workers provide an experimental basis for more recent hypotheses on the occurrence of an 'avalanche' reaction before the development of necrosis (Michalowski, 1981). Later studies (Hubbard & Hopewell, 1979) with similar, relatively high single doses (40 Gy) on the rat cervical spinal

cord showed somewhat different results, namely a steadily increasing population of oligodendrocytes during the first two months after irradiation, both in control and irradiated animals. At 3–4 months after irradiation, ~1 month before the occurrence of necrosis, the cell numbers dropped by almost 50%. Although the changes in cell numbers appeared to happen less drastically in the latter studies as compared to in those of Zeman *et al.* (1964), a similar trend in both studies is the substantial decrease in glial cell number before the occurrence of white matter necrosis. The control labelling indices in both studies were <1% effectively per 24 h. These results are compatible with the general consensus, primarily derived from the detailed studies by Korr, Schultze and co-workers (review, 1981) in murine brain, of a very slow turnover of target cells, with a growth fraction of less than 1%. An LI of 3% over 5 days labelling was reported by Hornsey *et al.* (1981) for the cervical spinal cord, but these authors concluded that proliferating glial cells had a long cycle time (~30 days). These conclusions were not based on direct cell kinetic measurements, such as differential labelling or grain counts, but on an interpretation of radiobiological results. Hornsey *et al.* (1981) observed a steady labelling index after a dose of 20 Gy, which is equivalent to ~65% of the ETD (as derived from published isoeffect doses for their rat model). A brief initial rise at ~3 weeks post-irradiation, could represent an abortive rise in proliferation in the glial stem cell compartment. After a rapid decline to control values, only at 120 days post-irradiation was a significant rise in LI again observed, which is in agreement with the recovery observed in the cervical cord during this period, and which is accompanied by an increase in latent period. Despite these differences in conclusions between various authors, most cell kinetic data are consistent with a very slowly turning over population of glial as well as endothelial cells.

At the relatively high isoeffect doses for the various functional and histological endpoints in the CNS, it is very unlikely that a substantial proportion of functional cells will divide more than once before the start of tissue breakdown. The occurrence of so-called 'subclonogenic' proliferation has been used as a theoretical argument to explain the wide shoulder on isoeffect survival curves for late tissue responses (Wheldon *et al.*, 1982). This hypothesis assumes, that doomed parenchymal cells pass through several divisions before they are lost from the pool of functional cells. This phenomenon would predominate after small doses. However, in slowly turning-over tissues, even extensive multi-fraction treatments are finished before any significant cycling of differentiated cells has occurred. Since most of the effects are scored at an isoeffect level of damage, it must then be assumed that the dose sparing observed in fractionated irradiations reflects intracellular repair. In addition, no significant change in latent period was observed at an isoeffective dose level for single dose or fractionated irradiations of the spinal cord. A longer latent period would have been expected when subclonogenic proliferation occurs after small doses.

A model of the radiation response of the spinal cord

From a synthesis of the above reviewed published work, and from new experimental results, a hypothesis on the role of target cell survival and repopulation in the spinal cord is schematically presented in Figure 5. In view of strong similarities between responses of cord and brain, the proposed model is expected to extend to the brain as well. The model will be more difficult to test in the brain because of the less specific endpoints available, especially in rodents.

The model is based on the following considerations and key-elements: Within a few weeks after irradiation, segmental demyelination is observed after doses as low as 5 Gy. This effect is the direct result of the loss of glial cells, and was observed to increase in frequency as a function of dose and time (Mastaglia *et al.*, 1976). It reflects the normally occurring cell loss in the absence of replacement by new cells. When a second dose of radiation is given during this interval (up to 2 months), the ED_{50} is equal to, or slightly lower as compared to a 2-fraction treatment with a 1 day interval. Also, no change in latent period, as measured from the first dose, is observed.

After about 2–3 months, the accumulated damage is sufficient to stimulate repopulation by oligodendrocytes. The start of repopulation may also depend on the time needed for a stem cell pool to recover and to produce a sufficient number of functional cells to offset the cell-loss. Qualitatively, this assumption is supported by the observation of remyelination starting at ~3 months p.i. (Mastaglia *et al.*, 1976). Similar observations on segmental demyelination and remyelination were made in lumbar nerve roots (van der Kogel, 1979 and unpublished results). Quantitatively, an increasing number of functional cells after 2–3 months is reflected by an increase in ED_{50} (white matter necrosis) and a lengthening of the latent period (at ED_{100} level) when a second radiation dose is given after 70 days or later. The ED_{50} increases further with longer intervals until it levels off at or before 140 days for the white-matter necrosis syndrome, at

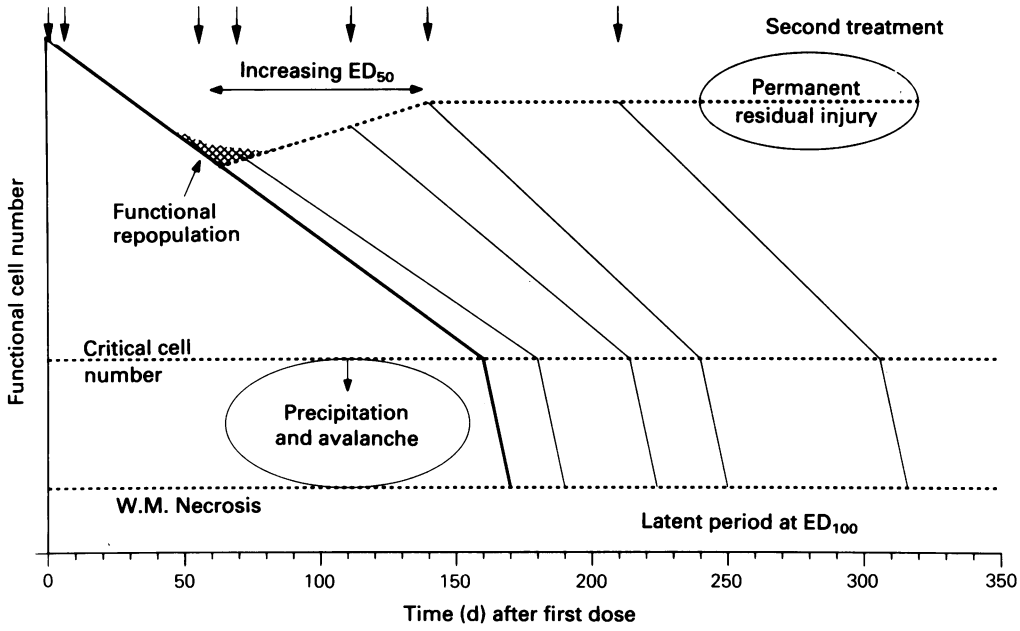

Figure 5 Schematic representation of a model for the development of white matter necrosis in the cervical spinal cord, as derived from results of split-dose treatments. The time of the second treatment is indicated on the upper X-axis. For further explanation see text.

a total dose equivalent to 135% of the reference ETD. The lack of further increase in ETD for the longer interval of 210 days, indicates a substantial amount of permanent residual damage in the cervical cord. The time-course of recovery may vary for different strains, and it is certainly different for the late vascular damage in the same strain (van der Kogel, 1982).

When the proliferating pool cannot compensate in time for the loss of functional cells, a critical number of glial cells will be attained and tissue breakdown may occur rapidly. This may be accelerated by abortive attempts at division by functional cells ('avalanche') or may be otherwise precipitated by damage in other target cell populations. It is obvious that, in the presence of different cell populations, one cannot analyze the role of one target cell without considering the damage induced in another population. The ultimate expression of tissue damage will depend on the interactions between the different tissue components. In the CNS, as in other tissues, this directly leads to the role of the vascular system, which in itself shows different types of radiation-induced damage over a period of weeks to years after irradiation (Law, 1981; Hopewell, 1983). In this respect it is of much interest that a shift in the expression of white matter necrosis and late vascular damage was observed in the rat cervical cord when the irradiated volume was reduced (Morris & Hopewell, personal communication). Decreasing the volume caused a significant increase in ED_{50} for white matter necrosis, but no change for the late vascular damage. The smaller contribution of vascular damage in the lumbar region (van der Kogel, 1979) may explain the more slowly developing nerve root necrosis (radiculopathy). The radiculopathy was microscopically detected to progress in a dose-dependent manner over a period of 1–2 months by a gradual loss of Schwann cells and segmental demyelination, starting at 3 months post-irradiation at ED_{100} dose levels (van der Kogel, 1979). After higher doses, a precipitating vascular damage cannot be excluded in the development of coagulation necrosis in the nerve roots (Bradley *et al.*, 1977).

An important element of the model is the observation of a highly reproducible latent period of 170 ± 10 days after irradiation of the cervical cord in WAG/Rij rats at the ED_{100} dose level. From the relationships between latent period and time interval between 2 fractions as given in (2) and (3), some assumptions can be made on the rate of cell loss. When a similar rate of cell loss is assumed at isoeffective doses for the various split-dose treatments, then reconstruction of the curves (as in Figure 5) suggests a constant number of functional cells at the time of the second fraction. In reality,

the isoeffect doses are observed to increase for time intervals beyond 70 days, up to a maximum value at 140 days. This suggests that the number of target cells increases from about 2 months p.i., and reaches a plateau at ~140 days. To explain the dose-latent period relationships at these higher isoeffect doses, an increased rate of cell loss has then to be assumed. Similarly, with equal isoeffect doses for a 140 and 210 day interval between 2 fractions, the shift in latent period is ~70 days, suggesting identical cell loss rates at equal isoeffect doses.

In the above description, an attempt is made to integrate the present knowledge of cell kinetics, pathology, and radiobiological observations on the late radiation responses of the CNS. It is realized that the proposed model is largely simplified and still far from an accurate representation of the mechanism of target cell depletion and the development of various late tissue effects in the CNS. Hopefully, the model will help in the design of further experimental studies to elucidate the role of various target cell populations in radiation-induced damage in the central nervous system.

The reported studies were performed in the Radiobiological Institute TNO, The Netherlands, and in the Los Alamos National Laboratory.

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